

Attorney Docket No.: DC-0301
Inventors: DeLeo, Joyce A.
Serial No.: 10/521,167
Filing Date: March 7, 2005
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Please amend the Specification as filed as follows:

At page 9, beginning at line 15, replace the paragraph with the following paragraph:

--RPA analysis of rat spinal cord tissue was also conducted and showed elevated MCP-1 mRNA at day 10 following surgery. Specifically, spinal MCP-1 mRNA in nerve-injured rats was 6.76 ± 2.41 times those levels in normal, unoperated rats; which reached statistical significance ($p=0.01$). The relative amount of mRNA for the MCP-1 receptor, CC chemokine receptor 2 (CCR2), using real time reverse transcriptase-polymerase chain reaction (RT-PCR) was also examined. Levels of CCR2 in rats with nerve transection were markedly elevated over sham and normal rats. CCR2 mRNA levels increased over normal levels as early as 4 hours following injury, reaching a peak six-fold increase at day 4. Similar results have been found in mice lacking CCR2 (Abbadle, et al. (2003) *Proc. Natl. Acad. Sci. USA* 100:7947-52). Further, the development of mechanical allodynia was totally abrogated in these CCR2-deficient mice. Sham levels at all time points of the studies conducted herein were not different from normal, yet injury produced significantly greater levels than observed for shams ($p<0.006$) at all time points following 4 hours. Allodynia patterns similar to those for the mice were observed for all rats receiving either sham or injury. ~~It has been suggested that CCR2 --~~

At page 10, beginning at line 31, replace the paragraph with the following paragraph:

-- The low dose of 4 μ g of anti-MCP-1 did not significantly alter behavioral hypersensitivity as compared to Hanks Balanced

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Salt Solution (HBSS) vehicle administration. However, at the higher dose of 20 ~~mg~~ µg of the MCP-1 neutralizing antibody, mechanical allodynia was significantly ($p < 0.001$) decreased for 12 gram von Frey stimulation. A similar response was observed for 2 gram stimulation ($p < 0.001$). These decreases were significant at days 7 ($p = 0.003$, 2 gm; $p = 0.002$, 12 gm) and 10 ($p = 0.002$, 2 gm; $p < 0.001$, 12 gm), despite terminating administration of the neutralizing antibody on day 5. Spinal MCP-1 protein levels were elevated over normal for all groups nerve-injured rats (**Table 6**). Moreover, no side effects were observed when anti-MCP-1 antibody was administered at either dose.--

At page 16, beginning at line 32, replace the paragraph with the following paragraph:

-- A customized RPA chemokine probe set (Pharmingen, San Diego, CA) for rats was utilized in a group of Holtzman rats to confirm mRNA changes in this species. Lumbar spinal cord tissue from L5 nerve-transected rats ($n = 5$) was harvested on day 10 following injury and spinal mRNA ~~leves was~~ levels were analyzed using RPA as disclosed herein. Tissue from normal animals ($n = 2$) was also included in the RPA analysis for comparison and normalization. Isolation of mRNA and RPA were performed according to the manufacturer's directions (Pharmingen, San Diego, CA). A customized template set was ~~uses~~ used to probe for the following chemokines and cytokines: MCP-1, IL-1ra, caspase-1, IL-18, MIP-2, IL-10, TNF- α , L32, and GAPDH (Pharmingen, San Diego, CA). Image analysis was performed using the IMAGEQUANT® software, version 5.2 (MOLECULAR DYNAMICS™, Sunnyvale, CA) and relative levels were compared between groups and reported as a (fold-increase over) ratio to normal levels.--